Is quinine a suitable probe to assess the hepatic drug-metabolizing enzyme CYP3A4?

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Aims To evaluate the antimalarial agent quinine as a potential in vivo probe for hepatic cytochrome P450 (CYP) 3A4 activity.

Methods Ten healthy adult volunteers received, by randomized crossover design, either a single oral dose of quinine sulphate (600 mg) alone, or quinine sulphate (600 mg) plus the CYP3A4 inhibitor troleandomycin (TAO; 500 mg every 8 h). Plasma and urine samples were collected before quinine administration, and up to 48 h thereafter, then analysed by h.p.l.c. for both quinine and its CYP3A4-generated metabolite, 3-hydroxyquinine. During both phases, the erythromycin breath test (ERMBT) was administered at specific times to assess hepatic CYP3A4 activity.

Results Compared with control, TAO treatment significantly decreased the mean time-averaged ERMBT result by 77% (95% CI, 68, 85%), the mean apparent oral clearance of quinine (CL/*F*) by 45% (95% CI, 39, 52%), and the mean apparent formation clearance of 3-hydroxyquinine ($CL_{3\text{-}OH}$) by 81% (95% CI, 76, 87%). There was no correlation between the TAO-mediated percent decrease in the time-averaged ERMBT result and the percent decrease in CL/*F* or in $CL_{3\text{-}OH}$. When TAO and control treatments were analysed separately, there were no significant correlations between the time-averaged ERMBT result and CL/*F*, $CL_{3\text{-}OH}$, or single plasma quinine concentration at 12, 24, and 48 h.

Conclusions Quinine may be a useful probe to detect inhibition of liver CYP3A4 activity within an individual. Further studies are needed to determine whether it can provide a quantitative measure of CYP3A4 activity suitable for intersubject comparison.

Keywords: CYP3A4, *in vivo*, metabolism, pharmacokinetics, probe, quinine

Introduction

In adult humans, cytochrome P450 (CYP) 3A4 is the most abundant CYP isoform expressed in small intestinal epithelial cells (enterocytes) and liver. CYP3A4 metabolizes a vast number of currently used drugs [1, 2]. There are large inter-patient differences in CYP3A4 content and catalytic activity in both liver and intestine [3–5]. This variability appears to account in part for inter-patient differences in dosing requirements of some CYP3A4 drug substrates. The prototypical CYP3A4 substrates cyclosporin and midazolam are such examples [6–8]. CYP3A4 also plays a potentially important role in the biotransformation of harmful dietary contaminants, including mycotoxins (e.g. aflatoxin B1), pyrizolidine alkaloids, and arylhydrocarbons (e.g. benzo[a]pyrene) [9, 10]. A logical but largely untested hypothesis is that genetic mutations leading to altered CYP3A4 gene expression may account for inter-individual differences in susceptibility to toxicity from these xenobiotics [11].

To evaluate this hypothesis, a means of measuring CYP3A4 activity in large numbers of individuals is required. It may also be desirable in some circumstances to measure selectively hepatic and not intestinal CYP3A4 activity, as intestinal CYP3A4 content can be greatly influenced by dietary manipulations. For example, consumption of a single glass of grapefruit juice causes a significant reduction in intestinal CYP3A4 activity but has little or no influence on hepatic CYP3A4 activity [12]. Dietary differences may explain in part why the relative activities of CYP3A4 in liver and intestine do not correlate within an individual [5]. Thus if the goal of a study is to identify individuals with genetically altered

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CYP3A4 activity, it might be desirable to measure selectively hepatic CYP3A4 activity only. To date, indirect measurement of hepatic CYP3A4 activity involves intravenous injection of the probe (e.g. midazolam or the erythromycin breath test). It would obviously be desirable to develop a safe oral probe that could selectively measure liver CYP3A4 activity.

Quinine is one of the most widely prescribed drugs for the treatment of resistant *Plasmodium falciparum* malaria [13]. It is also used for the treatment of leg cramps in the elderly. In healthy subjects, the absolute oral bioavailability of quinine is very high (~90%) [14], indicating that the drug is nearly completely absorbed into the systemic circulation following oral administration and undergoes little first-pass extraction. In addition, grapefruit juice had no effect on the oral pharmacokinetics of quinine [15], confirming that first-pass metabolism of quinine by the intestine is negligible. The fraction of a dose of quinine excreted unchanged in the urine, however, is low (12%), and only small amounts may be excreted into the bile [16], indicating that the drug undergoes extensive metabolism. This is most likely to occur in the liver and after it reaches the systemic circulation. Moreover, using human liver microsomes, it was shown that the metabolism of quinine to a major metabolite, 3-hydroxyquinine, is catalysed primarily by CYP3A4 [17, 18]. Based on these observations, we hypothesized that if hepatic CYP3A4 is the rate-limiting step in the elimination of quinine, this drug might serve as a safe and inexpensive oral probe for hepatic CYP3A4 activity.

This hypothesis was tested in healthy volunteers using two approaches. First, the effect of the mechanism-based, selective CYP3A4 inhibitor troleandomycin (TAO) [19, 20] on the disposition of quinine and 3-hydroxyquinine was examined. If hepatic CYP3A4 is rate limiting in the elimination of quinine, the apparent oral clearance of quinine and the apparent formation clearance of 3-hydroxyquinine should decrease significantly. Second, correlations were examined between hepatic CYP3A4 activity (as measured by the erythromycin breath test) and the following pharmacokinetic measures of quinine: apparent oral clearance, terminal elimination half-life, and single plasma concentrations at various times.

Methods

Chemicals and reagents

Quinine hydrochloride dihydrate was purchased from Fluka Chemical Corp. (Milwaukee, WI, USA). 3-Hydroxyquinine was a gift from Dr P. Winstanley (University of Liverpool, Liverpool, UK). Cinchocaine (used as an internal standard), sodium dodecyl sulphate, tetrabutylammonium bromide, hyamine (benzethonium) hydroxide and thymolphthalein were purchased from Sigma Chemical Co. (St Louis, MO, USA). The scintillation cocktail, Emulsifier-Safe®, was purchased from Packard Instrument Co. (Meriden, CT, USA).

Preliminary study in female cancer patients

To determine the appropriate timing of TAO dosing to maintain CYP3A4 inhibition *in vivo*, a preliminary study was conducted in three adult female patients with ovarian cancer (age, 45–71 years; weight, 53–96 kg). The University of Michigan Institutional Review Board approved the protocol, and written informed consent was obtained from all subjects. Each patient received a single oral dose of TAO (500 mg), and the ERMBT was administered at various times thereafter. To account for ^{14}C in breath from preceding tests, it was determined prior to injection and subtracted from the breath ^{14}C content determined 20 min later (see below for calculation of the test result). Since the ERMBT had never been administered on more than two occasions within a 24 h period prior to this study, there was concern that the kinetics of residual ^{14}C from earlier injections might create error in the test result after multiple tests. Therefore, only five tests were administered per patient.

Subjects and study design

Ten healthy volunteers, six women and four men, were recruited for the study. Four of the women and one man were African American, and the remaining five subjects were Caucasian American (two women, three men). Subjects' ages ranged from 23 to 61 years (mean ± s.d., 34 ± 11 years) and weight from 46 to 119 kg (78 ± 20 kg). Each subject was physically normal and had no history of past illness or hypersensitivity to any of the drugs used in the study. Before participating in the study, each subject underwent a routine physical examination, 12-lead ECG, a blood chemistry screen, and a complete blood count. No specific restraints were imposed on the subjects except for a 72 h abstinence from any caffeine-containing food and beverages, grapefruit products, and alcohol prior to the study day and 48 h after quinine administration.

The University of North Carolina Institutional Review Board approved this protocol, and each subject provided written informed consent prior to participation. A randomized crossover design was used, consisting of two phases, each separated by at least 2 weeks. Following an overnight fast, a baseline ERMBT was obtained. For the control phase, immediately following completion of the ERMBT, a single oral dose of 600 mg quinine sulphate (Zenith Pharmaceuticals, Miami, FL) was given

(time 0). Twenty-four and 48 h later, the ERMBT was repeated. For the TAO phase, immediately following completion of the ERMBT, 500 mg TAO (Pfizer, Inc., New York, NY) was given orally 2 h before quinine administration and every 8 h thereafter until the end of the 48 h period. An ERMBT was repeated immediately prior to quinine administration (i.e. 2 h after the first dose of TAO) and at 6, 24, and 48 h thereafter. During each 48 h phase, a blood sample (5 ml) was drawn from an indwelling catheter prior to and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 30, 36 and 48 h after quinine administration. Plasma was separated from blood cells by centrifugation. Urine samples were collected before and at 0–6, 6–12, 12–24, and 24–48 h intervals after quinine administration. Plasma and aliquots of urine samples were stored at −20 °C pending analysis for quinine and 3-hydroxyquinine.

Erythromycin breath test (ERMBT)

The ERMBT was performed as described previously [19]. Briefly, 3 μCi (0.01 mmol) of [^{14}C *N*-methyl] erythromycin (Metabolic Solutions, Nashua, NH) were diluted in 5 ml of 5% dextrose in water immediately before its intravenous administration. Twenty minutes after injection, the subject was instructed to exhale through a tube, creating bubbles into a solution of hyamine hydroxide:ethanol (50 : 50, v/v) to which a trace amount of the blue indicator, thymolphthalein (1%), had been added. When the blue dye turned clear, 2 mmol of carbon dioxide were trapped. Scintillation fluid (12 ml) was then added, and the specific activity of ^{14}C was measured by scintillation counting. ERMBT results are expressed as the percentage of the administered dose exhaled as $^{14}CO_2$ over the first hour after injection, as estimated by a single breath collection at 20 min [21].

Analytical procedures

Plasma concentrations of quinine and 3-hydroxyquinine were simultaneously quantified by a reversed-phase h.p.l.c. method using a Hewlett Packard (Palo Alto, CA) series 1100 system with fluorescence detection as previously described [22] with slight modification. In brief, the mobile phase consisted of an acetonitrile:phosphate (10 mM) buffer (50 : 50, v/v) containing sodium dodecyl sulphate (25 mM) and tetrabutylammonium bromide (3 mM), adjusted to pH 2.1 with orthophosphoric acid. Under this chromatographic condition, optimum separations of 3-hydroxyquinine, quinine, and internal standard (cinchocaine) were achieved. Retention times were 3.6, 6.4 and 8.3 min, respectively. Plasma and urine samples collected prior to quinine administration showed no endogenous sources of interference. The limits of quantification for quinine and 3-hydroxyquinine were 0.015 mg l^{-1} and 0.006 mg l^{-1}, respectively. Inter- and intra-day coefficients of variation in the assay for quinine and 3-hydroxyquinine were less than 6% at high (3–10 mg l^{-1}) and medium (0.5–2.5 mg l^{-1}) concentrations and 9.2% at the limits of quantification.

Pharmacokinetic analysis

Concentration *vs* time data for quinine and 3-hydroxyquinine from both treatment phases were analysed by standard noncompartmental methods with the aid of the software program WinNonlin (v2.1, Pharsight, Palo Alto, CA). The peak plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were obtained by visual inspection of the plasma concentration-time curve. The terminal elimination rate constant (λ_z) was determined by log-linear regression of at least the last four data points from the plasma concentration-time profile, and the terminal elimination half-life ($t_{1/2}$) was calculated as ln 2/λ_z. The area under the curve (AUC) from time zero to the last measured concentration (AUC(0,C_{last})) was determined using the linear trapezoidal method. The AUC from zero to infinite time (AUC(0,∞)) was calculated as the sum of AUC(0,C_{last}) and C_{last}/λ_z, where C_{last} is the last measured plasma concentration. The apparent oral clearance of quinine (CL/*F*) was calculated as the ratio of dose to AUC(0,∞). The fraction of the dose excreted unchanged in the urine ($f_{e,Q}$) or metabolized to 3-hydroxyquinine ($f_{e,3\text{-}OH}$) was calculated by dividing the cumulative urinary recovery (0–48 h) of quinine or 3-hydroxyquinine, respectively, by the dose. The renal clearance of quinine (CL_R) was calculated by dividing the 48 h urinary recovery of quinine by the AUC(0,48 h). The apparent formation clearance of 3-hydroxyquinine ($CL_{3\text{-}OH}$) was calculated as the product of $f_{e,3\text{-}OH}$ and CL/*F*.

Statistical analyses

All statistical analyses were performed using SAS (v6.10, SAS Institute, Cary, NC). All pharmacokinetic measurements are presented as the mean ± s.d. with 95% confidence intervals (95% CI). Differences in pharmacokinetic measurements between the control and TAO phases were assessed using the paired Student's *t*-test. Differences in the ERMBT result at the various time points were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons. The mean of the three (control phase) or four (TAO phase, post TAO administration) ERMBT results (see study design) was determined for each subject (i.e. a time-averaged ERMBT) and used as an index of hepatic CYP3A4 activity. The strength of the linear relationship between

the time-averaged ERMBT result (control or TAO phase) and the various pharmacokinetic measures was determined by Pearson's correlation (r). P values <0.05 were considered statistically significant.

Results

A preliminary study was conducted in three cancer patients to determine the optimal timing of TAO administration to maintain maximal inhibition of hepatic CYP3A4 activity. The maximum decrease in the ERMBT result occurred 2 h after TAO administration (Figure 1, Subject 1), and at least 70% inhibition was observed at 8 h (Subjects 1 and 2). CYP3A4 activity appeared to have recovered to baseline by 24–44 h (Subjects 1 and 3). Based on these results, the current study was designed such that TAO was given as a pre-treatment 2 h before the administration of quinine and every 8 h thereafter until the end of the study phase.

Shown in Figure 2 (*upper panel*) are typical ERMBT results, obtained at three (control) or five (TAO) different occasions over the 48 h study period, for a representative subject (Subject F). Also shown (*lower panel*) is the ERMBT result profile for the one atypical subject (Subject G). This subject experienced side-effects (nausea and headaches) from TAO after two consecutive doses and vomited after taking the third dose. Accordingly, TAO was discontinued, but the subject soon felt better, and the pharmacokinetic portion of the study was completed without further TAO treatment. During the control phase, the mean ERMBT result was unchanged over the time course (Table 1). In contrast, during the TAO phase, the mean ERMBT result significantly decreased by 95% (95% CI, 93, 98%) 2 h after the first dose of TAO and then increased at the end of the 8 h TAO dosing interval (i.e. 6 h after quinine was given) (Table 1). The mean ERMBT result then decreased at 24 h (i.e. 2 h after the TAO dose), but rose again at 48 h (also 2 h after the TAO dose) (Table 1). Exclusion of Subject G from the TAO phase did not change the mean ERMBT values at the first three time points (–2, 0, and 6 h relative to quinine administration; see Table 1) but did decrease the mean ERMBT values at the last two time points (to 0.25 and 0.82 at 24 and 48 h, respectively). TAO treatment significantly decreased the mean time-averaged ERMBT by 77% (95% CI, 68, 85%) (Figure 3). This value was essentially unchanged if Subject G was excluded (80%; 95% CI, 76, 85%). All tests of sta-

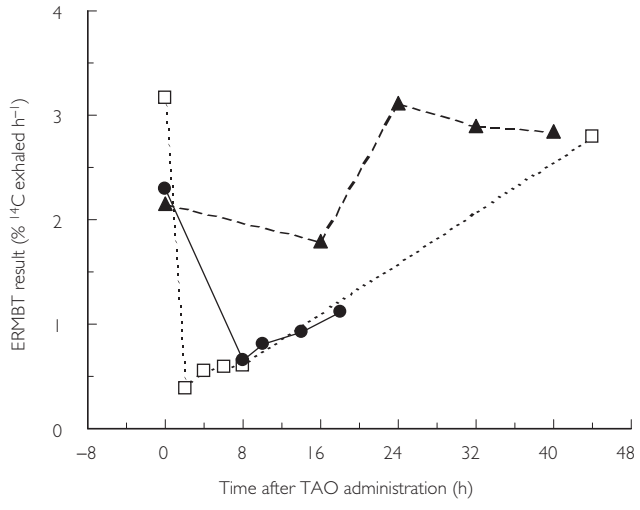

Figure 1 Time course of the effect of a single oral dose (500 mg) of troleandomycin (TAO) on hepatic CYP3A4 activity, as measured by the ERMBT, in three female cancer patients. □ subject 1; ● subject 2; ▲ subject 3.

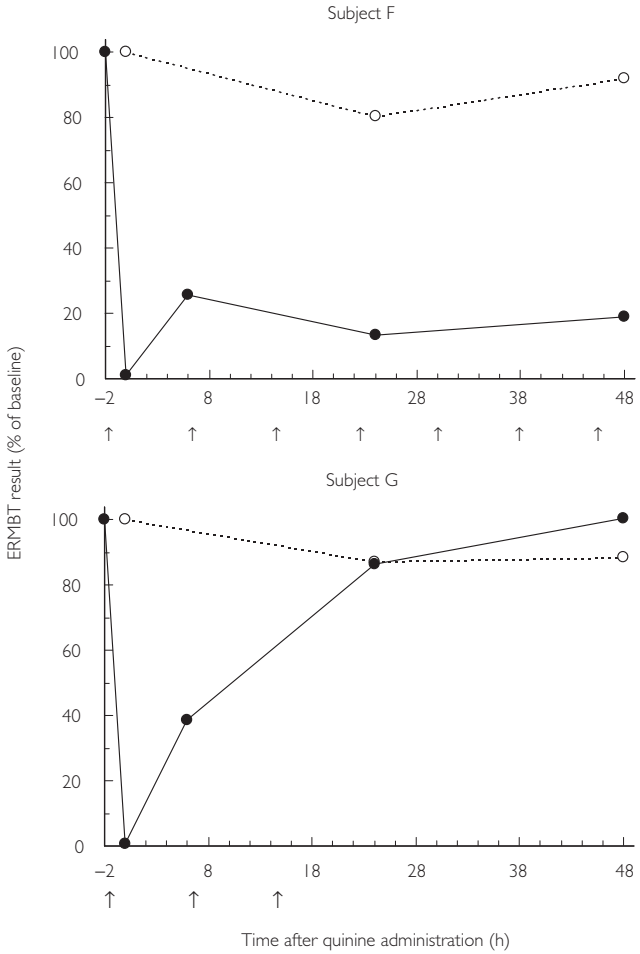

Figure 2 Time course of the effect of multiple doses of troleandomycin (TAO, 500 mg) on hepatic CYP3A4 activity (ERMBT) in a typical subject (Subject F) and in the one atypical subject (Subject G) in whom TAO was discontinued due to side-effects. ○ represents the control phase (quinine alone), ● represents the TAO phase (quinine +TAO), and arrows represent times when TAO was given.

Table 1 Time course of the ERMBT result obtained from 10 healthy subjects given quinine alone (control phase) or quinine +TAO (TAO phase).

	Time relative to quinine administration				
Subject	*−2 h*	*0 h*	*6 h*	*24 h*	*48 h*
Quinine alone			*ERMBT (% of ^{14}C exhaled h^{-1})*		
A		2.40		1.92	1.95
B		1.77		1.55	1.45
C		1.66		2.59	2.23
D		2.40		1.92	1.95
E		2.93		3.99	3.31
F		2.83		2.28	2.61
G		2.93		2.55	2.59
H		3.29		3.19	3.21
I		2.24		2.77	1.77
J		2.01		1.81	1.85
Mean ± s.d.		2.45 ± 0.54		2.46 ± 0.73	2.29 ± 0.62
95% CI		2.11, 2.78		2.00, 2.91	1.91, 2.68
Quinine +TAO			*ERMBT (% of ^{14}C exhaled h^{-1})*		
A	2.04	0.20	0.28	0.14	0.96
B	2.15	0.13	0.46	0.11	0.70
C	2.23	0.04	0.64	0.15	0.56
D	3.34	0.46	1.56	0.81	1.35
E	3.78	0.12	0.68	0.18	0.86
F	2.64	0.03	0.68	0.35	0.50
G	2.35	0.02	0.91	2.03	2.36
H	2.71	0.02	0.77	0.19	1.11
I	1.84	0.10	0.53	0.13	0.41
J	1.76	0.08	0.99	0.22	0.94
Mean ± s.d.	2.48 ± 0.65	0.12 ± 0.13★	0.75 ± 0.35★	0.43 ± 0.60★	0.98 ± 0.57★
95% CI	2.08, 2.89	0.04, 0.20	0.53, 0.97	0.06, 0.80	0.62, 1.33

★Significantly different from corresponding value at −2 h (ANOVA followed by Dunnett's test for multiple comparisons). Tests of statistical significance were unchanged if Subject G were excluded (see Results).

tistical significance remained unchanged with Subject G excluded from the analysis.

Representative plasma concentration *vs* time profiles for quinine and 3-hydroxyquinine after a single oral dose of quinine sulphate (600 mg) for the two treatment phases (control and TAO) are shown in Figure 4. The corresponding profiles for subject G from 24 to 48 h (i.e. when the ERMBT indicated no inhibition of CYP3A4) were not obviously different from those in the other nine subjects (not shown). However, there were only three data points to consider. Therefore, Subject G was included in the statistical analysis of the pharmacokinetic data.

With TAO treatment, quinine concentrations were higher, especially during the later time points, as compared to the control phase (Figure 4, *upper panel*). The formation of 3-hydroxyquinine was less during the TAO phase than in the control phase throughout the time course (Figure 4, *lower panel*). Pharmacokinetic measurements for quinine and 3-hydroxyquinine are summarized in Table 2 and 3, respectively. Values obtained from the control phase agreed with those previously reported for healthy volunteers [16, 23–25]. Compared with control, TAO significantly increased the mean quinine AUC(0,∞) and C_{max} by 90% (95% CI, 66, 113%) and 26% (95% CI, 16, 36%), respectively, and decreased the mean CL/*F* by 45% (95% CI, 39, 52%) (Table 2, Figure 3). In addition, TAO significantly prolonged the mean terminal elimination half-life ($t_{1/2}$) of quinine by 63% (95% CI, 49, 77%) and increased the mean percentage of the quinine dose excreted unchanged in the urine (0–48 h) by 101% (95% CI, 63, 138%). There were no significant differences in mean values for t_{max} and Cl_R of quinine between the control and TAO phases (Table 2).

During both phases of the study, 3-hydroxyquinine displayed formation rate-limited kinetics (Figure 4), as its average terminal elimination half-life (14.3 and 25.7 h for control and TAO phases, respectively) was not significantly different from that for quinine (12.3 and 20.0 h for control and TAO phases, respectively) (Tables 2 and 3). Compared with control values, coadministration of TAO with quinine resulted in significant decreases in the

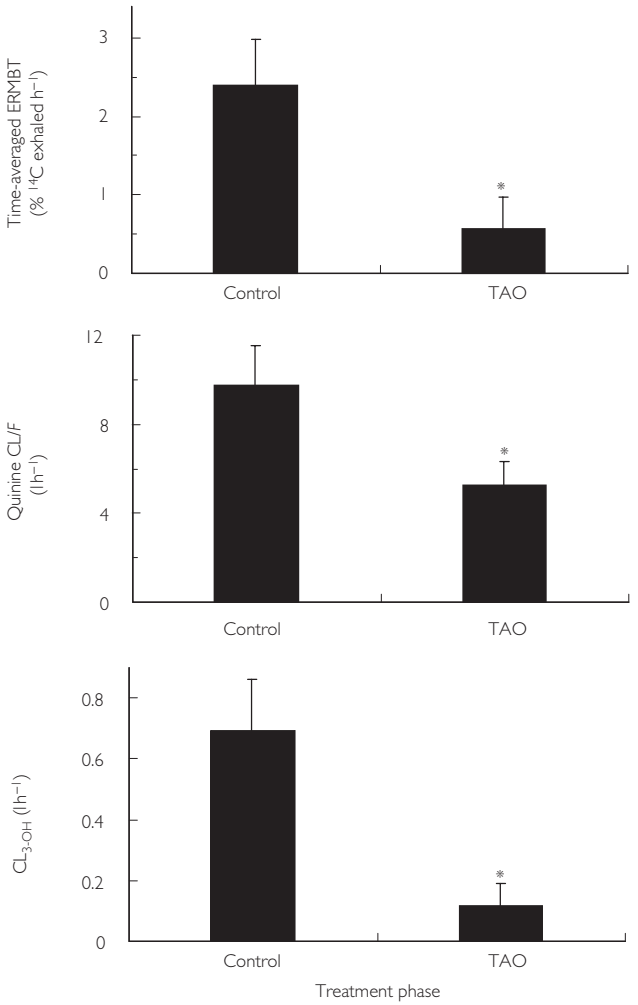

Figure 3 Effect of TAO treatment on the time-averaged ERMBT result (upper panel), apparent oral clearance (CL/*F*) of quinine (middle panel), and apparent formation clearance of 3-hydroxyquinine ($CL_{3\text{-}OH}$) (lower panel). Bars and error bars denote the average and standard deviation, respectively. Asterisks (*) indicate that the mean value from the TAO phase statistically differed from that in the control phase (paired Student's *t*-test, $P < 0.0001$).

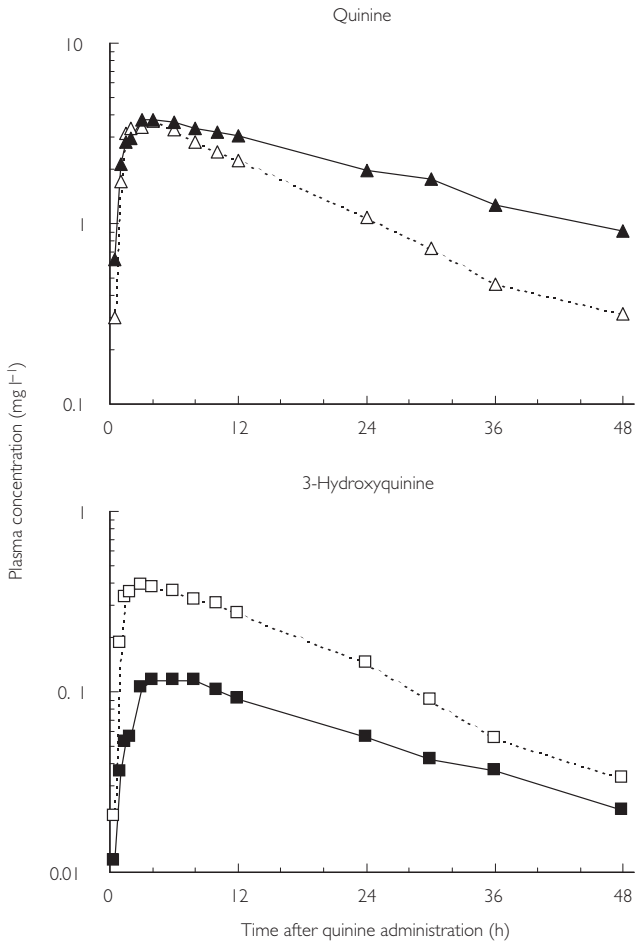

Figure 4 Plasma concentration *vs* time profiles of quinine and 3-hydroxyquinine following administration of a single oral dose of quinine sulphate (600 mg) to a representative subject (Subject B). Open symbols represent the control phase (quinine alone); solid symbols represent the TAO phase (quinine +TAO).

average AUC(0,∞) (58%; 95% CI, 51, 65%), C_{max} (75%; 95% CI, 70, 81%), the percentage of the dose excreted in the urine as 3-hydroxyquinine (65%; 95% CI, 55, 74%), and apparent formation clearance (81%; 95% CI, 76, 87%) of 3-hydroxyquinine (Table 3). The mean terminal elimination $t_{1/2}$ of 3-hydroxyquinine was significantly prolonged during the TAO phase. Although not significant, the mean t_{max} increased nearly three-fold (Table 3).

When data from the control and TAO phases were analysed separately, there were no significant correlations between the time-averaged ERMBT result and the following: CL/*F*; single plasma quinine or 3-hydroxyquinine concentration at 12, 24, and 48 h;

Table 2 Pharmacokinetics of quinine following a single oral dose (600 mg) of quinine sulphate to 10 healthy subjects during two treatment phases: control (quinine alone) and TAO (quinine +TAO). Data are presented as mean ±s.d. (95% CI).

Measure	*Control phase*	*TAO phase*
AUC(0,∞) (mg l^{-1} h)	63 ± 10 (57–69)	118 ± 27⋆ (102–135)
C_{max} (mg l^{-1})	3.2 ± 0.5 (2.9–3.4)	3.8 ± 0.6⋆⋆ (3.5–4.2))
t_{max} (h)	3.3 ± 1.8 (2.2–4.4)	3.6 ± 2.4 (2.1–5.1)
CL/*F* (l h^{-1})	9.8 ± 1.8 (8.7–10.9)	5.3 ± 1.0⋆ (4.6–5.9)
$t_{1/2}$ (h)	12.3 ± 1.7 (11.2–13.3)	20.0 ± 4.5⋆ (17.2–22.8)
$f_{e,Q}$ (0,48 h)	10.1 ± 2.4 (8.6–11.6)	19.6 ± 5.5⋆ (16.2–23.1)
CL_R (l h^{-1})	0.97 ± 0.21 (0.84–1.10)	1.03 ± 0.35 (0.82–1.25)

AUC(0,∞), area under the plasma concentration-time curve from zero to infinite time; C_{max}, peak concentration; t_{max}, time to reach C_{max}; CL/*F*, apparent oral clearance; $t_{1/2}$, terminal elimination half-life; $f_{e,Q}$, percent of the dose excreted unchanged in the urine; CL_R, renal clearance. ⋆$P < 0.001$; ⋆⋆$P < 0.01$ (paired Student's *t*-test).

Table 3 Pharmacokinetics of 3-hydroxyquinine following a single oral dose (600 mg) of quinine sulphate to 10 healthy subjects during two treatment phases: control (quinine alone) and TAO (quinine +TAO). Data are presented as mean ± s.d. (95% CI).

Measure	*Control phase*	*TAO phase*
AUC(0,∞) (mg l^{-1} h)	7.7 ± 3.1 (5.8–9.5)	3.4 ± 2.2⋆ (2.0–4.7)
C_{max} (mg l^{-1})	0.32 ± 0.11 (0.25–0.38)	0.08 ± 0.03⋆ (0.06–0.09)
t_{max} (h)	3.2 ± 1.8 (2.0–4.3)	9.4 ± 8.2 (4.3–14.5)
$t_{1/2}$ (h)	14.3 ± 2.6 (12.7–15.9)	25.7 ± 11.7⋆⋆ (18.5–33.0)
$f_{e,3\text{-}OH}$	7.1 ± 1.6 (6.1–8.2)	2.5 ± 1.2⋆ (1.7–3.3)
$CL_{3\text{-}OH}$ ($l\ h^{-1}$)	0.69 ± 0.17 (0.59–0.80)	0.13 ± 0.07⋆ (0.09–0.17)

AUC(0,∞), area under the plasma concentration-time curve from zero to infinite time; C_{max}, peak concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, terminal elimination half-life; $f_{e,3\text{-}OH}$, fraction of the dose excreted in urine as 3-hydroxyquinine; $CL_{3\text{-}OH}$, apparent formation clearance of 3-hydroxyquinine. ⋆$P < 0.001$; ⋆⋆$P < 0.01$ (paired Student's *t*-test)

$CL_{3\text{-}OH}$; and the metabolite-to-parent AUC ratio. There were also no correlations between the ERMBT result at 24 and 48 h and the corresponding plasma concentrations. When data from the two treatment groups were pooled, statistically significant correlations existed between the time-averaged ERMBT result and each of the aforementioned indices. However, these correlations resulted solely from the two distinct clusters created by control and TAO-treated subjects. Within subjects, there were no correlations between the percent TAO-mediated decreases in the ERMBT result and quinine CL/*F*, or between the percent decreases in the ERMBT result and $CL_{3\text{-}OH}$.

Discussion

Previous studies have established that TAO is a potent and selective inhibitor of human hepatic CYP3A4 *in vivo*, as shown by considerable reductions (>75%) in the ERMBT result [19] and systemic clearances of the CYP3A4 substrates midazolam and alfentanil [20]. The current study, utilizing the ERMBT, demonstrated that TAO administered every 8 h resulted in continuous potent inhibition of CYP3A4 activity throughout the entire 48 h period. The inhibitory effect appeared to abate slightly at the end of the first TAO dosing interval (i.e. 6 h after quinine was administered) (Table 1), suggesting that dosing intervals greater than 8 h would result in less inhibition. The inhibitory effect also appeared to abate between 24 and 48 h (Table 1). In rats, TAO treatment has been shown to increase CYP3A mRNA production and presumably CYP3A enzyme synthesis [26]. If this is also the case in humans, this mechanism could account for the gradual rise in CYP3A4 activity and may limit the usefulness of TAO for chronic inhibition.

During the TAO phase, the mean apparent oral clearance (CL/*F*) of quinine significantly decreased by 45% compared with control. Since quinine is essentially completely absorbed into the systemic circulation [14], and TAO had only a modest effect on mean C_{max} and t_{max}, the reduction in CL/*F* by TAO treatment was primarily due to inhibition of the systemic clearance of quinine. Therefore, because the mean renal clearance was also unchanged, the effect of TAO was likely to be on the hepatic clearance of quinine. The mean apparent formation clearance of 3-hydroxyquinine ($CL_{3\text{-}OH}$) was also significantly reduced by TAO treatment (81%), consistent with previous *in vitro* studies demonstrating that the formation of 3-hydroxyquinine is mainly catalysed by CYP3A4 [17, 18]. Thus, the present study demonstrates that hepatic CYP3A4 accounts, on average, for at least 45% of quinine CL/*F* and up to 81% of $CL_{3\text{-}OH}$. Correspondingly, this infers that up to 55% of CL/*F* may be non-CYP3A4 mediated. At least seven metabolites of quinine have been detected in human urine, with three of these being identified as 3-hydroxyquinine, 2′-oxoquininone, and quinine glucuronide [27]. The enzymes responsible for the latter two pathways are not known. The conclusion that hepatic CYP3A4 is a major pathway for the elimination of quinine is also consistent with the *in vivo* observation of a six-fold increase in quinine CL/*F* following pretreatment with rifampicin [25], a prototypical CYP3A4 inducer. However, it is possible, that intestinal CYP3A4-mediated metabolism could become important in the disposition of quinine after treatment with rifampicin (or other CYP3A4 inducers).

Mirghani *et al.* [28] reported that coadministration of quinine with the CYP3A4 inhibitor ketoconazole led to a significant decrease (31%) in both the mean quinine CL/*F* and 3-hydroxyquinine AUC(0,∞). The greater percent reductions observed in the current study suggest that TAO is more effective than ketoconazole as an inhibitor of CYP3A4 *in vivo*. It is also likely that TAO is a more selective inhibitor than ketoconazole, as the latter has been shown to inhibit the secretory (efflux) transporter P-glycoprotein (P-gp) [29–31]. Located on the apical (lumenal) surface of human enterocytes [32], P-gp acts to extrude its substrates from the interior of the enterocyte back into the intestinal lumen, often leading to a decrease in the fraction of the dose absorbed. There is considerable overlap between CYP3A4 and P-gp substrates [33], potentially rendering it difficult to distinguish the effects of ketoconazole on the two proteins. In contrast to ketoconazole, we have found that TAO does not inhibit P-gp in the human intestinal cell line Caco-2 [34]. Collectively, these observations suggest that TAO

may be superior to ketoconazole as an experimental means of selectively inhibiting human CYP3A4 activity *in vivo*.

It is not known whether quinine is hydroxylated by polymorphically expressed CYP3A5, which shares 84% amino acid sequence homology with CYP3A4 [35]. If so, variability in the expression of CYP3A5 may have influenced the changes in quinine disposition. Interestingly, half of the subjects were African-American, who have been reported to possess at least one CYP3A5 allele at a greater frequency compared with Caucasians [36]. TAO inhibits CYP3A5 to a much lesser extent compared to CYP3A4 (K.E. Thummel & D.J. McConn, University of Washington, personal communication). Therefore, if quinine were metabolized by CYP3A5, the TAO-mediated decrease in quinine CL/*F* in CYP3A5 expressors would be predicted to be less compared with nonexpressors. Since the current group of subjects was neither genotyped nor phenotyped for CYP3A5, the influence of CYP3A5 expression on quinine disposition cannot be ascertained at present.

There is large inter- and intra-individual variation in human CYP3A4 activity, between 10-fold and 40-fold, depending on the method used and the population studied [4, 5, 7]. This large variability in CYP3A4 activity accounts for much of the inter- and intra-patient variations in the disposition of many CYP3A4 substrates [6]. The search for a suitable probe (biomarker) to assess hepatic CYP3A4 activity has therefore been intense. Many CYP3A4 probes have been proposed, including the ERMBT [19], the ratio of endogenous cortisol to 6β-hydroxycortisol [37], and midazolam systemic clearance [7, 8]. The utility, suitability, and limitation(s) of each have been discussed elsewhere in detail [6, 38].

It was anticipated that results from the current study would substantiate the use of quinine as the first oral probe capable of quantifying hepatic CYP3A4 activity. In support of this, following TAO treatment, both the mean CL/*F* of quinine and $CL_{3\text{-}OH}$ decreased in parallel with the mean time-averaged ERMBT result (Figure 3), suggesting that quinine may be suitable to assess inhibition of hepatic CYP3A4. However, within an individual, there were no correlations between the percent decrease in either quinine CL/*F* or $CL_{3\text{-}OH}$ and the percent decrease in the ERMBT result. In addition, time-averaged ERMBT results from the control or TAO phase did not correlate with either quinine CL/*F* or $CL_{3\text{-}OH}$. The absence of such correlations may not necessarily mean that no correlation existed, as the sample size (10) could only detect a strong correlation ($r > 0.8$) with 85% probability. It should also be noted that the different CYP3A4 probes generally fail to correlate with one another, and multiple factors may account for these lack of correlations [39, 40]. Future studies will examine whether quinine CL/*F*, or other surrogates, can predict the CL/*F* of other CYP3A4 substrates, such as midazolam.

In summary, using TAO as a chemical 'knockout' for CYP3A4, we have confirmed that a major pathway for the metabolism of quinine *in vivo* is mediated by hepatic CYP3A4. Compared with ketoconazole, TAO appears to be the experimental treatment of choice to ablate CYP3A4 activity *in vivo*. Because CYP3A4 plays a prominent role in quinine elimination, quinine may serve as a useful probe to assess within-subject inhibition of liver CYP3A4 activity in certain circumstances. For example, this approach can be easily performed in patients already receiving quinine as treatment or prophylaxis for malaria. Further studies are needed to determine whether quinine can provide a quantitative measure of CYP3A4 activity suitable for intersubject comparison.

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